ON THE FLAGELLATES OCCURRING IN THE INTESTINE OF *GLOSSINA PALPALIS* AND IN THE INTESTINE AND PROBOSCIS OF *GLOSSINA MORSITANS*

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1. INTRODUCTION

The following notes form a record of some observations which were made on the parasites occurring in the intestine of *Glossina palpalis* and in the intestine and proboscis of *Glossina morsitans*. They are admittedly scanty and incomplete, as we were able to devote only a very short time to the work, and much of this time was disadvantageously employed through the non-receipt of literature bearing on what had already been done on these protozoa.

In the earliest efforts made to trace a developmental cycle of the pathogenic trypanosomes in the tsetse flies, e.g., in the work of Koch,[1] and Gray and Tulloch,[2] the not unnatural mistake was made of considering all the parasites found in the flies to be derived from the pathogenic forms. As a result of further work done in conjunction with Minchin,[3] Gray and Tulloch admitted that their earlier conclusions were erroneous, and about the same time Novy[4] pointed out the possibility of the same thing. Stuhlmann[5] took the precaution of working with laboratory-bred flies, but in a recent paper, in which the whole subject is reviewed, Patton and Strickland[6] consider that sources of error exist in his experiments, and they also take exception to some of Minchin's[7] results and to those of Keysselitz and Mayer,[8] Roubaud,[9] and others.

In the light of our present knowledge of the wide-spread infection of many arthropods by various protozoal parasites there are some grounds for the criticism of much of the previous work. It is therefore apparent that in approaching a subject beset by so many inherent difficulties the greatest care must be exercised in the conduct of the work and that it must be controlled in the most complete manner. The use of 'wild' tsetse flies in work on development cannot be justified on any ground; for, apart from the presence of benign parasites which confuse the results, there exists a possibility that they may be naturally infected with a different species of trypanosome from the one which is being employed in the experiments. Even in the case of laboratory-fed flies, the criticism has been made that sufficient care has not been observed to exclude the occurrence of a hereditary transmission of the non-pathogenic parasites. Minchin and Stuhlmann dispute the possibility of this, while Novy and McNeal and Patton and Strickland think that it may occur.

II. CONDITIONS UNDER WHICH WORK WAS DONE

Our observations, in the case of *Glossina palpalis* (var. *wellmani*), were carried out on Matondwi Island at the southern extremity of Lake Tanganyika. This island lies between two and three miles from the mainland, and measures about one mile in length by half a mile in width. It slopes with more or less abruptness from the water to a height of about one hundred feet, and is composed of rough broken stones on which grows a thin scrubby type of bush. The shores are similar to those of the lake in that they present at some places dense growths of reeds, at others scattered mimosa trees, and at others bare rocky stretches devoid of vegetation. *Glossina palpalis* was very abundant along the shore, and large numbers were caught daily without any difficulty.

For more than the past twenty years the island has been uninhabited, and during this period has practically never been visited even temporarily by the natives of the mainland. The fauna is composed of some crocodiles, large numbers of birds, principally water-species (e.g., darters, egrets, herons, &c.), a few snakes, and a species of mouse; no other mammalia exist, save perhaps an occasional visiting hippopotamus.

During the month of July, 1908 (middle of dry season) we spent four weeks on Matondwi, and in November, 1908 (just after the rains had commenced), returned for a three days' visit.

The work with *Glossina morsitans* was done at a camp near Kambole, about 50 miles west of Abercorn, during the month of October, 1908 (end of dry season). In this immediate neighbourhood, game is very scarce at that time of the year. Only an occasional duiker was seen while we were at this place.

III. METHODS

We found the best method of extracting the intestine was as follows.

The fly was laid, dorsal surface downwards, on a clean slide, so that the last segment nearly touched a large drop of salt-citrate solution (NaCl and Sodium Citrate ā ā 0·5 per cent.) in the centre of the glass. A mounted needle was then pressed against the posterior surface of

the thorax, and traction was made on the everted hypopygium in the male, or on the last abdominal segment of the female, with a second needle. The last segment was thus separated from the rest of the abdomen and with it the attached gut was pulled out into the drop of fluid. With a little care the whole intestine, including the oesophageal portion, could be obtained intact and quite free from the other abdominal contents. The malpighian tubules were usually the only other organs which came away with the gut; sometimes spermathecae, ovaries, and uterus. This procedure, as will be seen, varies slightly from that given by Minchin,[7] in which the terminal segment of the fly was snipped off and the whole of the abdominal viscera expressed.

The whole intestine was mounted in the salt-citrate solution and examined under the low and high powers. Fresh preparations and smears were afterwards made from the various portions of the intestine and other abdominal contents. The smears were fixed in absolute alcohol and Fleming's solution and stained by Giemsa's and Leishman's methods.

The proboscis was examined in salt-citrate solution, and smears were made and stained as in the case of the intestinal contents.

IV. INCIDENCE OF INTESTINAL CONTAMINATION

A. *Glossina palpalis.*

During July, 1,409 *Gl. palpalis* were caught, 1,282 males and 127 females. Of these, 185 were dissected and examined, and 78 were found to harbour intestinal protozoa, a percentage of 42·1. This is by far the highest percentage of tsetse flies which has yet been recorded as containing these trypanosomes. In the Sesse Islands 11 per cent. were infected, in the French Congo about 10 per cent., in the Congo Free State infection is apparently unknown, according to Dutton, Todd and Hanington.*[10]

The comparative ratio of infection in the two sexes was approximately the same.

	Positive	Negative	Percentage
Males	73	96	43·1
Females	5	11	37·5

* This is rather questionable, however, judging from the extremely widespread occurrence of the condition.

It was observed also that the ratios of infection amongst batches of flies caught in various sections of the island were markedly different, e.g.:—

Section	Positive	Negative	Percentage
A	47 ...	40 ...	54
B	7 ...	22 ...	24·1
C	24 ...	45 ...	34·9

No apparent reason existed for these discrepancies. Section A, the most heavily infected, was directly opposite the camp, and our natives were constantly going and coming from the water along this stretch, so that it was avoided by all the birds. No crocodiles were ever seen along this bit of the shore.

About a third (33·1 per cent.) of the flies which were examined showed evidence of having fed on blood (61 out of 185). In 25, oval, nucleated cells were seen; in 35, the pigment and detritus left from digested blood; and in only one, fresh mammalian blood was found, evidently from one of the boys engaged in catching the flies.

In November, 401 flies were caught, 396 males and 5 females. One hundred of these were examined, 99 males and 1 female, and 41 were found to be infected.

	Positive	Negative	Percentage
Males	41 ...	58 ...	41·4
Females	0 ...	1 ...	0

At this time special attention was devoted to the proboscis, but in no case were parasites found in this part of the flies.

B. *Glossina morsitans.*

Of 365 flies, 313 were males and 52 females, a proportion of 6 : 1. One hundred and thirteen were examined, and of these 32·8 per cent. showed signs of having fed on blood (in 3 mammalian blood corpuscles were observed, and in 34 blood pigment), practically the same percentage as noted in the case of *Gl. palpalis.* All traces of blood disappear from the intestine of *Gl. morsitans* in about 96 hours, and it is altogether probable that, if the flies feed on blood alone, they are capable of remaining alive without it for some considerable period. The condition of the abdominal organs of many of the flies which were examined was such as to render it perfectly clear that much longer than four days had elapsed since last they had fed on blood.

The possibility of tsetse flies feeding on anything except blood has recently been mooted again.[11] The following observation, though negative, may be worth noting.

On one occasion at the mouth of the Kalambo river, a tributary of Lake Tanganyika, numbers of *Gl. palpalis* were seen to settle at the edges of little puddles of stagnant water containing various low forms of plant life. We could not determine that they were sucking up the water, and at the time were not in a position to examine the flies.

Nine of the 113 *Gl. morsitans* were found to be infected with protozoa, i.e., 7·8 per cent. This intestinal infection apparently tends to die out, or at all events, the parasites do not retain the ordinary trypanosome form after a few days, as is evidenced by the following:—

Time of examination	No. Examined		
	Male	Female	Percentage
Freshly caught	31 (5 positive)	2 (negative)	15·1
24 hours after capture ...	60 (4 ")	6 "	6
48 " " " ...	2 (negative)	0	0
96 " " " ...	12 "	0	0

V. INCIDENCE OF INFECTION IN PROBOSCIS

As stated above, infection of the proboscis of *Gl. palpalis* with the parasites was never observed. It was found to be present in seven out of thirty-one *Gl. morsitans*, 21·2 per cent. In contradistinction to the intestinal infection, that of the proboscis apparently increases in frequency on keeping the flies, e.g.:—

Time of examination	No. examined	No. infected	Percentage	Condition of intestine in infected cases
Freshly caught ...	8	1	12·4	Parasites present
24 hours after capture	10	4	40	Parasites present in 3, absent in 1
48 " " "	3	2	66·6	Parasites present in 1, ? in other, fly lost
96 " " "	12	0	0	

Taken in conjunction with the figures given above, this may indicate, as Stuhlmann[5] suggests, that the intestinal infection gradually works forward, and that the proboscis is infected last. If the intestinal forms really die out in two or three days, the reinfection might be explained by some of the proboscis forms, which are almost always present in the hypopharynx, being ingested with a succeeding feed.

VI. MORPHOLOGY OF FORMS FOUND IN *GL. PALPALIS*

In fresh preparations, as in stained ones, the parasites presented a great diversity of size and shape, from extremely long narrow forms to short broad ones. One variety had a body somewhat like the section of a bi-convex lens, with a long, lash-like flagellum; a second variety was a very narrow, rod-like organism, which moved with considerable rapidity; a third variety was 'beaked,' that is, the posterior portion was narrow, and succeeded by a more or less central bulbous portion which tailed off into the flagellum. The development of the undulating membrane varied from nothing, to one which was fairly-well defined. The two types, long thin ones and short clubbed ones, could almost always be seen, though, of course, many intermediate forms between these two were also present. In those flies in which the parasites were few in number, they were as a rule of the broad short type. In the majority of cases the trypanosomes were extremely numerous, and in a few instances large clumps of them were seen adhering by their flagellate extremities to the gut wall towards the distal end of the intestine, similar to the clumps described by Novy, McNeal and Torrey[12] in mosquitoes. The trypanosomes composing these clumps moved slowly from side to side. In addition to the free forms carrying flagella, rounded motionless parasites devoid of flagella were also observed in fresh preparations. These are apparently derived from the 'broad' type of organisms.

In only one fly were the parasites observed outside the true intestine. In the one referred to, a few were seen in the preparation from the proctodaeum. This fly had been fed on a dog about an hour previously.

Trypanosomes, or any bodies recognisable as derived from them, were never seen in the salivary glands, malpighian tubules, testes, fat bodies, ovaries, or other organs of the flies.

Stained preparations.

The description is given almost entirely from the slides which were examined in Africa. Like Minchin, we have found that the ones which were left to be stained at home have proved to be practically useless.

In general, the trypanosomes may be divided into the two types, long narrow ones, and broad shorter ones, which have been mentioned above.

The long type of organism is chiefly met with in two separate forms. The first of these measures from 30μ to 40μ in length by $1{\cdot}7\mu$ to 2μ in width. The posterior extremity is usually rounded or bluntly angular, while the anterior, or flagellar extremity, is more or less acutely drawn out. The body width is uniform throughout the greater part of the length of the parasite, so that it bears some resemblance to a narrow strip of ribbon. The nucleus is oval; as a rule occupies the whole width of the body, and does not present any peculiarities of structure other than in a few instances; being composed of a central, deeply-staining portion, from which projects at either pole a more loosely-built wing. The blepharoplast is large, oval or round, and is usually placed in close apposition to the nucleus. More generally its position is anterior to the nucleus, but it may be lateral or posterior, and in some instances is separated by an appreciable interval from it. The undulating membrane, as a rule, is not well marked, and may not be present. The flagellum varies from examples in which a distinctly free portion of some length is present, to those in which none is discernible. A slight club-shaped thickening of the terminal portion of the free flagellum is not uncommon, and in one or two cases the root is expanded into a fan-like arrangement close to the blepharoplast. The cytoplasm stains a darkish blue (Giemsa's stain), and may contain a few small granules, though commonly it is free from these, and is quite homogeneous in composition.

The second variety of the long forms is an extremely narrow and elongated one, which may measure as much as $53\ \mu$ in length by only $0{\cdot}5\ \mu$ in width. The posterior extremity is acute, while the anterior end is drawn out very gradually along the flagellum. The nucleus is long, and occupies the whole width of the body. No definite

structure could be made out in it. The blepharoplast is rounded, and is usually superimposed on the nucleus. There is no visible undulating membrane, and the flagellum is prolonged into a well-defined free portion. The protoplasm stains a light blue, and is free from granulations.

This type of parasite is not nearly so frequent as the one previously described. They were seen in preparations made from a fly which had fed forty-eight hours beforehand on a hen, and from one which had fed an hour earlier on a monkey, but in this case they were not so long as the ones from the hen-fed fly. The broad forms of the organisms were also present in these preparations.

The more usual form of the broad type approaches in shape that of the ordinary blood trypanosomes. They measure from 19μ to 30μ in length and $1{\cdot}8\mu$ to 3μ in breadth. The posterior extremity is more or less rounded; the anterior end is attenuate. The nucleus is oval, and stains homogeneously. The blepharoplast is rounded or oval and occupies much the same position in relation to the nucleus as in the long forms. The undulating membrane is usually perceptible. The flagellum does not show the tendency to become clubbed, which was noted in the other variety. The protoplasm stains a light blue or pink, and may contain some granules.

Various modifications of the broad type exist, and the 'beaked' kind is probably one of them. In this form, the posterior extremity instead of being rounded in the ordinary manner, is elongated, so that the expanded nuclear area occupies a central position between two attenuated portions. The posterior extremity is not, however, extended into a filiform portion like the anterior end, but is usually cut off squarely. In other respects it is much like the ordinary broad type.

In most of the preparations, oval or rounded forms measuring about 4μ or 5μ in diameter, and without a flagellum, were present. The protoplasm stains darkly, and may contain granules. These forms are apparently derived from the ordinary broad ones. The posterior portion of the parasites containing the nucleus and blepharoplast become globular, while the anterior part loses its staining powers, becomes irregular in shape, and degenerates. The flagellum disappears coincidentally. No definite retaining wall is present, so that these forms are not true cysts. Encystment, as described by Minchin,[7] has not been observed in our preparations.

While the formation of the rounded forms may be traced, the reverse process, development into flagellated parasites, may also be seen from bodies very similar to Leishmania. The flagellum appears first as a comparatively short structure, and gradually increases in length, the body of the trypanosome becoming stretched out at the same time to assume the more ordinary character.

Division was seen in organisms of the broad type alone. Ordinary simple fission is the commonest, but instances occur in which a triple division is present.

Many gradations between the long and broad forms are present; probably immature specimens of these types. The appearances of all the forms agree with the description of *Trypanosoma grayi*, Novy, and to this category we would refer the parasites we have seen in *Glossina palpalis*. No forms recalling *T. tullochi* were observed.

VII. MORPHOLOGY OF FORMS FOUND IN *GLOSSINA MORSITANS*

A. IN INTESTINE. *Fresh examination.*

(1) Long filiform forms, of approximately the same width throughout the greater part of the body, but tapering gradually towards the flagellar extremity.

(2) 'Beaked' forms, with a narrow posterior portion, succeeded by a more or less oval central mass, and then tapering gently towards the anterior end.

(3) Blunt or clubbed forms, with a rounded posterior portion, which is the widest part of the body.

(4) Regularly oval or ovoid forms, with a flagellum apparently devoid of any undulating membrane, projecting from the narrow pole.

(5) Motionless oval or rounded forms devoid of flagellum.

The first four varieties are motile. The 'beaked' forms are rather peculiar, in that the posterior and bulbar portions always remain quite rigid, while the anterior portion is vibratile. This is explained by the position of the blepharoplast close to, and ordinarily in front of the nucleus, which lies in the expanded middle part of the parasite.

In all cases the parasites were much more abundant in the posterior part of the midgut. They were never observed outside the intestinal tract, and never in the proctodaeum.

Stained preparations.

The long forms measure from 26μ to 35μ in length and from $1 \cdot 8\mu$ to 2μ in width. Both ends are more or less acute, but the anterior is the more attenuated of the two. The nucleus is about 4μ to $4 \cdot 5\mu$ in length, is oval, and usually occupies the whole width of the body. The blepharoplast is relatively small, and in contradistinction to the forms seen in *Glossina palpalis*, is usually posterior to the nucleus, and is separated by an appreciable interval from it. The undulating membrane is very poorly developed, and the flagellum does not, as a rule, attain to any great length. The general protoplasm stains a rather deep blue, and is homogeneous in structure.

The clubbed variety measures 20μ to 25μ in length by about 3μ in width. The posterior extremity is rounded, and the body attains its maximum width a short distance anterior to this point. From the point of greatest width it tapers gently to the anterior end. The nucleus is rounded or oval, and is situated more towards the posterior part of the body. The blepharoplast, in these forms, frequently is placed anteriorly to the nucleus, and is usually small. The flagellum and undulating membrane are not well marked, and the free part of the flagellum is quite short. The body protoplasm stains rather lightly.

The 'beaked' forms are of comparatively frequent occurrence, but do not present any other peculiarity of structure than the special shape of the posterior part of the body. This has a rounded end, and is very narrow until it approaches the nuclear area when the body expands into an oval portion, beyond which it becomes gradually drawn out towards the anterior end.

The oval, flagellated forms when stained are seen to be composed of a large globular mass, from one end of which the body is prolonged as a narrow process containing the flagellum. The enlarged portion measures from 7μ to 15μ in length by $4 \cdot 5\mu$ to 6μ in width, and the prolonged portion from 6μ to 12μ. The nucleus is usually oval, and stains uniformly. The blepharoplast is rounded, and is usually in

close connection with the nucleus, either slightly anterior to or superimposed on it. No definite undulating membrane is present, and the flagellum is co-terminous with the prolonged portion of the parasite. The protoplasm may contain a few granules, and usually stains a uniform and rather deep blue.

The oval or rounded motionless forms are, except that they are devoid of flagellum, practically identical with the ones just described, which are apparently derived from them by the growth of the flagellum and coincident lengthening of the body. As in the case of similar forms seen in *Glossina palpalis*, no trace of a cyst wall is present.

Division forms are present amongst parasites of the broad type.

The parasites in the intestine of *Glossina morsitans* recall closely those of *Glossina palpalis*, the only marked difference being the almost uniformly posterior position of the blepharoplast, and the greater tendency to acuity in the posterior part of the body. These differences are not sufficiently decided, and the amount of work we were able to devote to the study is too small to enable us to say that they are a different species.

B. In Proboscis.

Fresh preparations.

In the proboscis of freshly-caught flies the parasites were found, as noted by Roubaud,[9] adhering to the labium and labrum in large clumps, and occasionally so numerous as apparently to occlude the lumen. Moreover, we observed them frequently within the hypopharynx also, in extremely large numbers. In both situations, the clumps, which resembled a chrysanthemum, were formed of very many parasites which had the flagellar extremity directed towards the walls of the proboscis. They moved with a slow wave-like action, and also by contraction. Single, separate organisms were fairly active, and, as described by Roubaud frequently became attached to the slide or to bits of tissue in the preparation. As a rule, they were present throughout the whole length of the proboscis, though variations were noticed from a decided massing of the parasites at the distal extremity to a scanty occurrence at the proximal end.

The parasites, in the fresh, appeared to be of various sizes and shapes. Some were almost filamentous, others were attenuated at either extremity, at the flagellar end more markedly so, while others again approached more closely the ordinary shape of the pathogenic trypanosomes. They appeared to be about one and a half times the diameter of the proboscis in length.

Parasites were generally present in the gut at the same time as in the proboscis, although this was not invariably the case. In at least one, no trypanosomes were seen in the intestine.

Stained preparations.

A special description of the forms seen in the proboscis is unnecessary, as they correspond closely to those observed in the gut. In general their dimensions are less, but in other respects the description of the various intestinal types applies equally well to those of the proboscis.

The beaked variety was the commonest, and after that the ordinary broad club-like forms. The long ribbon-like type was not seen in stained preparations, though a few forms resembling on a small scale the very narrow long type were observed. In many of the forms the flagellum did not stain at all, and, if present, was extremely rudimentary.

VIII. FEEDING EXPERIMENTS

These were originally undertaken in order to trace, if possible, a development of the 'wild' trypanosomes in *Glossina palpalis.* Many different sources of food were used, mammalian and avian, and the

Source of food	Flies fed	Flies examined	Positive	Present	
Dog	6	4	0	0	
Monkey	42	38	4	10·5	11·1 %
Guinea-pig	2	2	0	0	
Sheep	20	19	3	15·7	
Hen	17	17	2	11·6	
Hornbill	3	3	1	33·3	22·7 %
Darter	1	1	1	100	
Small, canary-like bird ...	1	1	1	100	

intestinal contents of the flies were examined at various periods afterwards, from a few hours to several days. The results of this examination were rather surprising, for whereas 42 per cent. of unfed (artificially) flies contained flagellates, only a very small proportion of the fed ones were found to harbour parasites.

The discrepancies shown by these figures are rather interesting, and rather difficult to explain, unless it be due to the fact that mammalian blood exerts some unfavourable action on the parasites in these flies. As we have already said, mammalian blood does not form any part of the ordinary food-supply of the flies on the island. The difference in the results between the flies fed on the hen and those fed on birds inhabiting the island is also suggestive. However, the results are so scanty that no great weight can be laid on the suggestion we have offered.

In Uganda, Minchin, Gray and Tulloch[3] found that goats' serum acted deleteriously on the 'wild' parasites, and found that by this means they could be distinguished from *T. gambiense.* Other sorts of serum had, however, no such action.

The following experiment was made to ascertain whether crocodile serum was more favourable to the parasites than that of some of the local birds.

Hanging drop preparations were made from the intestinal contents of a fly containing very many parasites, and were diluted in the proportion of 1 : 1 with salt-citrate solution and serum obtained from a crocodile, a hen and a darter. The preparations were examined at intervals of fifteen minutes for a period of four hours. The changes which occurred, consisted of a clumping of the parasites with the flagella towards the periphery, and a gradual cessation of the movement of the clumped forms. Simultaneously they started to break down, until finally there was left a granular and refractile mass. The clumping occurred rather more slowly in the preparation containing crocodile serum than in the other two, but otherwise precisely similar changes occurred, and at the end of four hours there were only a few free trypanosomes left.

A few *Glossina morsitans* were fed on a dog. In one series ten flies were fed, and examined at intervals of from one and a quarter to three hours later. None of them contained parasites. In another series eleven were fed on the same dog, and examined forty-eight

hours later. Two of these flies contained intestinal parasites. In one they occurred as small clumps with the flagellar ends directed towards the periphery, and in the other as long freely motile forms. Parasites were also present in the proboscis of this latter fly.

IX. ORIGIN OF PARASITES

Some conflict of opinion exists as to the origin of these 'wild' parasites. It would seem that, to a certain degree, this may be explained by the views held by various writers on the question as to the food of tsetse flies. Novy,[4] who thinks that they may possibly feed on other substances than blood, is of the opinion that the parasites may possibly be derived from stagnant water, or some similar fluid. Reference has already been made to an instance in which the flies may have been imbibing such a fluid. Against this, however, is the experience in raising and maintaining tsetse flies in captivity, which, so far, has shown that blood is the only food which they will ingest.

On the other hand, Minchin[7] and others consider that blood alone is the natural food of the tsetse flies, and that in all probability the parasites are derived from a vertebrate host, possibly a bird. To a certain extent, the infection may be due to the 'contaminative' transmission, that is the ingestion of the cystic forms of *T. grayi*, though the probability of the continuation of the fly-infection in this way is rather slight.

So far, no vertebrate host of these parasites has been discovered. On the island we examined the blood of darters, pigeons, hornbills, several species of small birds, snakes, mice and crocodiles (4), but in none of them were parasites of any description seen. *T. sp.* (?) were seen in frogs caught on the mainland.

The difference of opinion on the question of hereditary transmission also tends to complicate the solution. Patton and Strickland,[6] who incline to this hypothesis, quote in support of their view the instance in which Minchin, Gray and Tulloch[3] observed the infection in a laboratory raised fly which had been fed on a hen. If hereditary transmission of the flagellates, and food other than blood are eliminated, it is rather difficult to account for the infection of so large a percentage of *Gl. palpalis* on the island. It may be

argued that if hereditary transmission does occur, all the flies in such a restricted area should have been infected. As is known, the infection tends to die out of the intestine, or at least apparently so, though the persistence of unrecognised forms of the parasites cannot yet be excluded. This, however, depends on the lack of suitable pabulum, and it is within the bounds of possibility that when a 'negative' fly does get a meal of the required sort, the ordinary forms of the flagellates may be evolved from the unrecognised forms. In the flies we examined, traces of a meal (blood) were found in 33·1 per cent. and parasites in 42 per cent., but as the blood is digested in a shorter period of time than the parasites take to disappear from the intestine, this discrepancy can be accounted for. To some degree, the reinfection of the intestine may be due to ingestion of the proboscidal forms, provided that the fly feeds a second time before these have died out.

The further point also arises as to the identity of the forms seen in *Gl. morsitans*. In general they appear to be similar to those found in *Gl. palpalis*, except in minor points. If they are the same species, many species of vertebrates must harbour the parasites, if we accept the view that they are derived from vertebrates, since *Gl. morsitans* feeds to a great degree on a different fauna from that on which *Gl. palpalis* feeds. There is no reason to suppose that the two species are identical other than the similarity in form, and this is not a decisive feature for the determination of species amongst the trypanosomes.

Diversity of opinion also exists as to the origin of the forms found in the proboscis. Stuhlmann[5] considers that they are derived from the intestinal forms, the infection of the proboscis being the final stage in the development of ingested trypanosomes. Roubaud,[9] on the other hand, thinks that the infection in the proboscis is an entirely distinct thing and that it is to be accounted for by the fixation and multiplication of pathogenic trypanosomes, *in situ*, which have been ingested in the act of feeding.

There are several points which tend to invalidate Roubaud's conclusions. He does not state whether he examined the proboscides of freshly-caught, unfed flies for the presence of flagellates, and it is possible that he mistook the naturally-occurring forms for those

developed from pathogenic ones. If they are derived from these, and not from the ordinary intestinal forms, there does not seem to be any reason why the development in the proboscis should only occur in 10 per cent. of the flies fed, and not in all, and this is the percentage in which intestinal infection was present in his flies. Moreover, he does not give any reason for stating that the intestinal parasites are cultural forms of *T. pecaudi* which must have been derived from big game. In the first place, it is extremely rare to find trypanosomes in buck, and, secondly, it does not follow that such parasites as may be present are *T. pecaudi.* We do not wish to criticise unnecessarily, but in working with tsetse flies great caution must be observed before reaching definite conclusions. The greatest objection to the assumption that the forms seen in the proboscis are derived from pathogenic trypanosomes is that they are innocuous when injected into susceptible animals.

Our results are discordant, for in *Gl. morsitans*, both intestinal and proboscidal forms were encountered, while in *Gl. palpalis* only the first were seen. This may be accidental, and more continued work might have resulted positively.

Of the two hypotheses we are inclined to agree with that of Stuhlmann: that the parasites in the proboscis are derived from those in the intestine.

It is, perhaps, possible that the parasites occurring in 'wild' tsetse flies, as distinct from laboratory bred ones, may be derived from pathogenic trypanosomes which lose for some unknown reason their infectivity when ingested, and it is possible that the intestinal forms do not all belong to one species but represent a mixed infection. This can only be decided by lengthy experiments.

In the case of *Gl. morsitans* it would seem on *a priori* grounds that a development of the pathogenic trypanosomes does occur, for it is a reasonable certainty that any susceptible animal exposed to their bites in nature will become infected. All such infections cannot be explained by a mechanical transmission. The recent work of Kleine, and its confirmation by Bruce, shows that a development apparently does occur.